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LATENT POLYPHENISM AND DIRECT DEVELOPMENT IN
PIERIS VIRGINIENSIS (PIERIDAE)

Additional key words: diapause, hostplant, Brassicaceae.

In many pierids, seasonal polyphenism in wing pattern is coupled with diapause (Oliver
1970, Shapiro 1976), which is facultatively inhibited by rearing larvae under long-day
photoperiods and warm temperatures (*P. rapae* L., Barker et al. 1963; *P. napi* L., Lees
and Archer 1981; *P. napi oleracea* Harris, Oliver 1970). Populations of *Pieris napi* may
contain individuals with varying response to natural photoperiod (Shapiro 1976), as well
as individuals whose diapause lasts for several years (Shapiro 1979). Unexpected envi-
ronmental stress such as unseasonably cold weather can induce more individuals to enter
carryover diapause, ensuring the population's survival during extreme but short-lived
environmental changes (Shapiro 1979). Yet another pierid diapause pattern is one in
which all members of the population diapause and the insect is univoltine.

Pieris virginiensis Edwards is a monophagous species that uses an ephemeral, vernal
hostplant, toothwort, *Cardamine diphylla* (Michx.) A. Wood (= *Dentaria diphylla* Michx.).
Matching the restricted growing season of its host, *P. virginiensis* is a univoltine species
that enters diapause when reared at summer photoperiods that inhibit diapause in *P.
napi oleracea* (Shapiro 1971). Facultative diapause has been observed in several *P. vir-
giniensis* populations, however. Forbes (1960) refers to a rare second brood of *P. virgi-
niensis* occurring in Massachusetts and West Virginia, but does not give specific localities.
Bowden (1971) reared 3 non-diapausing offspring from a stock of *P. virginiensis* sent to
him by S. A. Hessel presumably from Washington (Litchfield Co.), Connecticut.

Shapiro (1971) obtained direct-developing *P. virginiensis* by subjecting the larvae to
continuous light at 25°C. While the normal adult phenotype of *P. virginiensis* resembles
a smokey version of the heavily veined vernal form of *P. n. oleracea,* Shapiro found that
non-diapausing *P. virginiensis* adults were indistinguishable from the summer form of
P. n. oleracea, a phenotypic similarity also noted by Forbes (1960). Shapiro concluded
that the mechanism for phenotypic polyphenism was still intact but latent in *P. virgi-
niensis.* We report here on a population of *P. virginiensis* producing direct-developing
individuals whose offspring we have crossed in the laboratory.

Pieris virginiensis occurs together with *P. n. oleracea* in beech-maple-hemlock woods
near Lee, Massachusetts (Berkshire Co.). Large stands of toothwort are present in early
spring and are followed by garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande
(= *A. officinalis* Andrz.), as the season progresses. By mid-July the toothwort population
has senesced; only garlic mustard remains in abundance along with small stands of
watercress, *Rorippa nasturtium-aquaticum* (L.) Hayek (= *Nasturtium officinale* R. Br.),
and cuckoo-flower, *Cardamine pratensis* L. Together these crucifers could support a
sizable summer brood of either species. Courant et al. (1994) present evidence of incipient
host range expansion of *P. n. oleracea* to garlic mustard, but there is no evidence that *P.
virginiensis* is able to exploit garlic mustard successfully (Bowden 1971, our unpublished
data) although they oviposit on it in the field (Roger W. Pease, Jr. personal communication,
Courant et al. 1994, Porter 1994).

On 7 May 1993 we collected from this site seven female *P. virginiensis* which laid
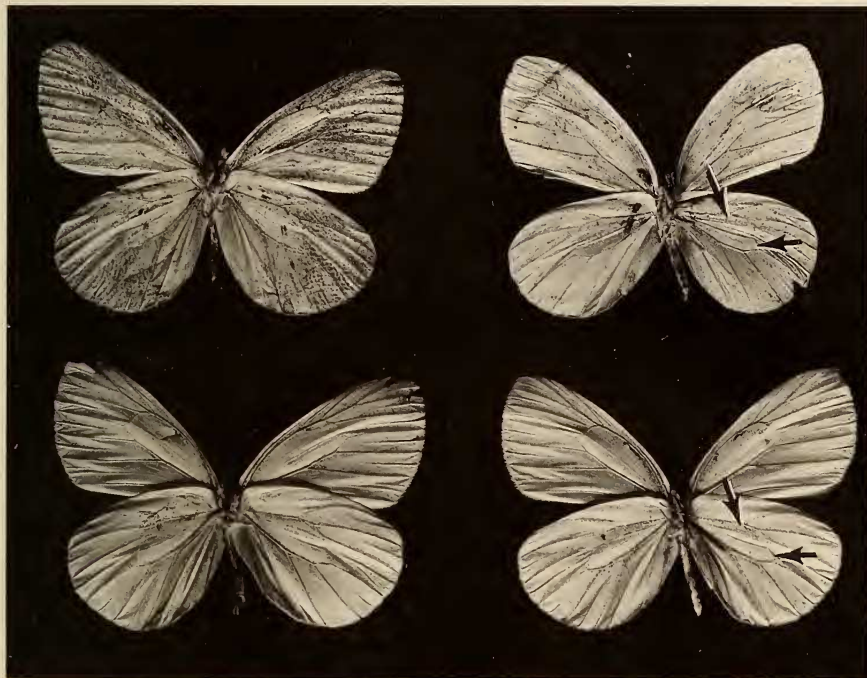
FIG. 1. Ventral view of direct-developing lab-reared offspring from field-caught females of *P. virginiensis* and *P. n. oleracea* near Lee (Berkshire Co.) Massachusetts. Top row from left: *P. virginiensis* ♂, *P. virginiensis* ♀. Bottom row from left: *P. n. oleracea* ♂, *P. n. oleracea* ♀. All were reared under long day conditions (16L:8D; 27°C day:19°C night) and eclosed within 10 days after pupation. Arrows show where LHW color was sampled in the discal cell near the origins of veins Rs and M_3.

eggs in our laboratory. Eggs hatched 11–20 May and larvae were reared as one combined brood under long-day laboratory conditions (16L:8D, 27°:19°C) on a variety of crucifers. On 21 May 1993 the larvae began to pupate. We noted two pupal colors, brownish white as described by Edwards (1888), and green which included a phenotype closely resembling non-diapausing *P. n. oleracea*, as well as intermediates between brown and green. The pupae were kept in our laboratory (room temperature: 22–26°C) with the intent to later chill them to break diapause.

Of the approximately 80 *P. virginiensis* pupae, 8 male and 8 female adults emerged between 1–21 June 1993, eclosing from green or intermediate pupae, although not all green or intermediate pupae developed directly. Possibly these 16 were siblings, because the offspring of all 7 wild-caught females were reared together. Like the non-diapausing *P. virginiensis* observed by Shapiro (1971) and Forbes (1960), the first generation of lab-reared offspring resembled the pale yellow summer form of *P. n. oleracea*, except the ground color of the wing was very white (Fig. 1). Melanic markings varied on the costal margin of the forewing and on the outer edge of the hindwing, but wings lacked the characteristic vernal melanic markings along the veins.

Three matings were observed among these 16 direct-developing *P. virginiensis* adults. One female died soon after mating without laying eggs. Eggs from the other mated females were reared separately under the above long-day conditions. The second lab-reared generation had higher than normal larval mortality in the first three instars; of 36

TABLE 1. Diapause and direct development in a second lab-reared generation of *P. virginiensis*. Shown are the diapausing and non-diapausing male and female offspring from 2 females that developed without diapause under long-day laboratory conditions. Females A and B were mated to direct-developing males which were also from the first generation of lab-reared offspring from wild-caught *P. virginiensis* mothers.

	Diapausing	Non-diapausing	Lost/died	Total number
Female A				
♂	1	1	1	3
♀	0	4	1	5
Female B				
♂	1	2	2	5
♀	1	6	0	7
Totals				
♂	2	3	3	8
♀	1	10	1	12

third instar larvae, only 20 pupated (12 ♂ and 8 ♀). The same variation in pupal colors was noted. Both matings produced direct-developing and diapausing individuals (Table 1).

To quantify HW ground color, we took pictures of the LHW underside of lab-reared (summer phenotype) *P. n. oleracea* (9 ♂, 9 ♀) and direct-developing *P. virginiensis* (14 ♂, 11 ♀ from 2 lab-reared generations) using a video camera recorder (SONY CCD-TR81) with incandescent lighting (standardized using Kodak R-27 gray card). We input these pictures into a Macintosh computer and Adobe Photoshop 2.5 (Adobe Systems, Mountain View, CA). Two ground color samples were taken from the discal cell (Fig. 1) and characterized for red, green, and blue color components for which higher numerical values correspond to whiter hues. The color sample values from each specimen were averaged. Because these values were not normally distributed, they were compared using a one-tailed Mann-Whitney test (Zar 1984). All three color components in *P. virginiensis* are equal to or higher than their counterparts in the *P. n. oleracea* sample (Mann-Whitney $U'_{[18,25]} = 340$ for red [p= 0.0023]; $U'_{[18,25]} = 311.5$ for green [p = 0.033]; $U'_{[18,25]} = 337.5$ for blue [p = 0.0056]).

Non-diapausing individuals of *P. virginiensis* may occur for two reasons. First, the facultative diapause expressed in members of the *napi* group may be latent in *P. virginiensis* (Shapiro 1971). This explanation is consistent with observations that other populations of *P. virginiensis* contain direct-developing individuals (Bowden 1971, Forbes 1960). Lack of a suitable summer hostplant would limit the success of non-diapausing *P. virginiensis* in Connecticut. However, the site at Lee, Massachusetts, contains small stands of watercress, an acceptable hostplant for *P. virginiensis* (Bowden 1971, our unpublished data) that may permit flight season expansion similar to that observed by Shapiro (1975) in a Sierra Nevada population of formerly univoltine but now partially bivoltine *P. napi microstriata* Comstock.

A second explanation for direct-developing *P. virginiensis* is hybridization with *P. n. oleracea*. These two species occur in sympatry in Lee, Massachusetts; the middle of the *P. virginiensis* brood in early May coincides with the first emergence of *P. n. oleracea*. An interspecific mating (*oleracea* ♂ × *virginiensis* ♀) was observed near this site, but was infertile (Chew 1980). Hybridization experiments with these species, however, have produced viable offspring (Bowden 1971). Lorković (1986) also reports a successful interspecific hybridization, but does not report on offspring survival. Introgression of "facultative diapause" alleles into *P. virginiensis* by hybridization is problematic because the F_1 hybrid, which would emerge in June, cannot backcross with *P. virginiensis* after the *P. virginiensis* flight season ends in mid-May. However, our direct-developing *P. virginiensis* offspring could be F_1 hybrids. Where *P. virginiensis* and *P. n. oleracea* are

sympatric, a second brood of *P. virginiensis*, or an F_1 brood, might easily be missed because it closely resembles *P. n. oleracea* summer phenotype.

Considerable evidence suggests that expression of lepidopteran diapause is under genetic control (Shapiro 1976, Danks 1987), although Powell (1987) did not find convincing evidence for genetically fixed expression of prolonged diapause in his review of some 90 lepidopteran species. We have demonstrated that crosses of direct-developing *P. virginiensis* yield both direct-developing and diapausing individuals. Our small numbers and relatively high mortality/loss rate in the second lab-reared generation make it difficult to determine transmission genetics and further investigation is needed.

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